IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: WANG Application No.: 09/870,353 Filed: May 30, 2001 For: IMPROVED NUCLEIC ACID MODIFYING ENZYMES	Examiner: Richard Hutson Technology Center/Art Unit: 1652 DECLARATION BY DR. YAN WANG SUBMITTED UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Yan Wang, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

2. I am the inventor of the above-referenced application. I received a Ph.D. in Biochemistry from the University of Oregon in 1992. I am presently employed by Bio-Rad Laboratories, Inc as an R&D Manager/Principle Scientist in the Department of System Integration. My responsibilities include leading the Reagent Development R&D group in developing amplification reagents. I have held this position and similar positions for over six years. A copy of my curriculum vitae is attached as Exhibit 1.

3. I have read and am familiar with the contents of the application. As I understand the outstanding rejection, the Examiner believes that the pending claims are overly broad. Specifically, the Examiner contends that it would take undue experimentation to identify members of the genus of sequence non-specific double-stranded nucleic acid binding proteins that have at least 75% identity or at least 85% identity to Sso7d (or Sac7d), or at least 90% identity to Sac7d, and that can enhance processivity of a polymerase. The Examiner has now cited three publications that allegedly further support the rejection. In the Office Action, the references are characterized as showing that a single point mutation in Sso7d can affect the function of the nucleic acid binding domain.

4. It is the intent of this declaration to illustrate how the experiments performed in the cited publications in fact support the position that one of skill can successfully employ the rich structural Sso7d/Sac7d data available in the art to predict the effects of sequence changes on Sso7d/Sac7d function. In the cited references the authors were seeking to investigate Sso7d by introducing mutations that were predicted, based on the structure, to negatively affect function. Their results validated this approach. In the current invention, the skilled artisan can use this same structural information to reasonably predict sequence changes that preserve Sso7d/Sac7d function rather than destroy it. Each of the references is individually discussed below.

5. Wang, *et al. Nucl. Acids Res.* 32:1197-1207, 2004 ("Wang")

The Examiner points to Wang as further supporting the rejection because Wang teaches that a change in Trp24 of Sso7d significantly reduces the effectiveness of the protein in enhancing processivity. Wang is a post-filing publication of my work relating to polymerases that are modified by linkage to an Sso7d protein. In one aspect of the experiments presented in this article, we determined that Sso7d double-stranded DNA (dsDNA) binding activity is important for processivity, as taught in the current application. The interactions between Sso7d and dsDNA have been extensively studied. Trp 24 was identified in structural studies to be important for binding to dsDNA, as explained on page 1201, column 1 in the last paragraph. (Trp24 in Wang corresponds to Trp23 in SEQ ID NO:2 of the application as filed.) The

referenced structural studies (Gao *et al., Nature Struct. Biol.* 5:782-786, 1998; and Catanzano, *et al. Biochemistry* 37:10493-10498, 1998) were readily available in the art before our invention. We purposefully selected Trp 24 for mutation to further investigate the correlation between DNA binding and processivity. We created three mutant Sso7d-polymerase fusion proteins in which Trp 24 was replaced with Val, Gly or Glu, with the intent of reducing the ability of Sso7d to bind dsDNA and in turn, reduce its ability to enhance the processivity of the DNA polymerase. All three mutant fusion proteins exhibited decreased processivity relative to that of the wildtype Sso7d-polymerase fusion (see, the first column of page 1201 bridging to the second column), just as we expected. Substitution of Trp 24 with Glu, which we expected to exhibit the greatest effect because it differs the most from the wild-type residue, also resulted in the greatest decrease in processivity. I note that all three mutant Sso7d proteins still retained some ability to enhance processivity when compared to the unmodified polymerase (Table 2, page 1202).

This experiment shows how one of skill in the art makes use of structural information to recognize amino acid residues that are expected to be relevant to function. In our case, we intentionally selected a residue based on available Sso7d structural data with the expectation that we would compromise the function of Sso7d in enhancing polymerase processivity. This is precisely what we observed. As noted above, the same structural information can be used to select residues that would not be expected to alter Sso7d activity, which would be the goal in designing Sso7d variant proteins for use in the invention.

6. Consonni *et al., Biochemistry* 38:12709-12717, 1999 ("Consonni")

Consonni is cited by the Examiner as providing evidence that the claims are not enabled because a single amino acid change (Trp 23 or Phe 31) in Sso7d can alter function. However, Consonni also provides another example of how structural information is predictive of the functional importance of particular amino acid residues. This paper describes the solution structure of an Sso7d mutant protein F31A, in which an alanine is substituted for a phenylalanine residue at position 31. In prior studies cited in Consonni at page 12710 in the second full paragraph of the first column, Phe 31 was selected for mutation on the basis of structural data that indicated that this residue is located at the core of the aromatic cluster and has tight contact

with side chains of several residues in the cluster. This residue was therefore predicted to be important for stability. I note that this residue is also highly conserved in Sso7 family members, as can be seen in a sequence comparison of Sso7d, Sac7d, Sac7a, and Sac7e (see, the Rule 1.132 Declaration by Peter Vander Horn of record in this application) As the authors expected, the mutation of Phe 31 to Ala led to a loss in thermo and piezostabilities (third paragraph of column 1, page 12710). The analysis presented in the current Consonni paper relates to the solution structure of the F31A mutation, which was performed in order to determine the structural changes that were associated with the loss of stability of the mutant protein.

Consonni observed that in the solution structure of the F31A mutant, the Trp 23 residue was reoriented such that it pointed inside the aromatic cluster. Given the previously identified role of Trp23 in contacting DNA (Trp 23 is the same residue as Trp24 in Wang), the authors investigated the DNA-binding activity of the mutant F31A protein. The results showed that the binding activity was also impaired, once more highlighting that Trp 23 plays an important role in DNA binding, as indicated by the structure.

Consonni again provides an example of how a practitioner in this art makes use of structural information. With regard to the loss of stability observed in the F31A mutant protein, it is not surprising that the mutation affected Sso7d stability. It is well known in the field that an amino acid with a large, buried hydrophobic side chain stabilizes conformation. It is predictable that changing the large hydrophobic side chain to a small side chain would result in a loss of stability. It is therefore standard practice in this art to avoid radically mutating such residues, if it is desired to preserve function, just as it would be desirable to avoid mutating those residues that directly contact DNA to preserve DNA binding function.

7. Shehi, *et al. Biochemistry* 42:8362-8368, 2003 ("Shehi")

Shehi performed studies examining the thermal stability and DNA binding activity of Sso7d. I will first review the results of these analyses and then address the specific issues raised by the Examiner.

Shehi structural analysis

Shehi investigated the function of the C-terminus of Sso7d. The authors explain that structural properties of Sso7d were known and provide a brief description of the topology of Sso7d on page 8362. Shehi created an Sso7d protein that was truncated at Leu54 (L54Δ) in order to investigate the role of the C-terminal α-helix on stability and DNA binding activity. The region targeted in Shehi does not contact the DNA in the structural analysis of Sso7d and Sac7d DNA binding interactions. To determine whether deletion of the C-terminal region had effects on DNA binding, the authors analyzed the binding of L54Δ to double-stranded calf thymus DNA in comparison to the binding activity of wildtype Sso7d. It was found that the association constant for binding of L54Δ to double stranded DNA was similar to that of Sso7d (page 8362 bridging to page 8363 and Figure 4). Thus, deletion of the eight residues at the C-terminus of Sso7d did not result in loss of DNA binding activity, which was predictable based on the structure.

The authors also observed that a variant that was truncated at Glu 53 could not be isolated under the same conditions that allowed them to isolate L54Δ and noted that this highlights the role that Leu 54 plays in the folding process. Shehi explains that Baumann and colleagues (*Nat. Struc. Biol.* 1:808--809, 1994)) in fact described that the side chain of Leu54 is packed well against that of Ala50, anchoring the C-terminal end of the chain to the protein core. Other investigators also confirmed that Leu54 is involved in strong van der Waals interactions with the remaining part of the protein. Thus, the available Sso7d/Sac7d structural data provided information on the role of Leu 54 that was born out by the studies in Shehi.

Shehi's results are consistent with the analysis of Sso7d structure provided by Dr. Vander Horn in his Declaration that is of record in this application. Dr. Vander Horn indicated that in the context of DNA binding activity, the alpha helix is highly mutable, as evidenced by the fact that natural variation of Sso7 homologs is observed in this domain. Dr. Vander Horn cautioned, however, that the naturally occurring mutations in this domain appear to preserve the alpha helix. Thus, in designing Sso7d variants for use in the invention, one of skill would introduce mutations that preserved structure. I further note that the L54 residue is also conserved

across the naturally occurring Sso7 proteins, which also would be an additional consideration in designing variants with the purpose of retaining DNA binding activity.

Examiner's rejection

Shehi mentions that there were difficulties in isolating the deletion in which the C-terminus was truncated at Glu53 under the same conditions that were used to isolate L54Δ. Shehi also noted that L54Δ has a limited solubility in aqueous solution. The Examiner contends that "both mutations demonstrate the unpredictability of the effect of point mutations in Sso7d on any particular function or attribute of Sso7d." However, one of skill cannot conclude from the experiments in Shehi that the effects of point mutations at Glu53 or L54 would be unpredictable. Shehi investigated deletion mutations, not point mutations. The effects observed in deleting most of the C-terminal α-helix therefore cannot be extrapolated to the effects of introducing point mutations into that region. Again, in generating functional variants, one of skill would employ structural information that is available and information about the conservation of sequences/structure among family members in considering potential changes to an amino acid sequence.

In terms of the limited solubility of L54Δ, the authors believe that this is likely due to the loss of three net charges and the exposure of hydrophobic moieties upon deleting the last eight residues. It is recognized in the art that changing the charge of a protein and exposing hydrophobic residues can influence solubility. The ordinary artisan can additionally consider such effects in designing variant Sso7d sequences. I further note that Shehi was examining L54Δ alone, not when fused to a polymerase protein. The limited solubility observed by Shehi under these conditions would not necessarily reflect the solubility when the protein is fused to a polymerase.

8. In summary, the references cited by the Examiner repeatedly demonstrate that the structural information about Sso7d sequence and function provided a sound basis for accurate prediction of effects of Sso7d mutations on DNA binding function. In the current invention, in order to generate Sso7d variants that retain DNA binding function, and accordingly, the ability to

enhance processivity, the practitioner would use the same structural information to avoid those residues that participate in DNA binding function and have the same reasonable expectation of success.

Yan Wang

Dated: 7/5/2007

attachment: Exhibit 1

60984796 v1

Exhibit 1

YAN WANG, Ph.D.

EXPERIENCE:

2003-present *Principle Scientist/R&D Manager, Dept. of System Integration, GXD. Bio Rad Lab.(Employer change due to acquisition.)*
Lead the Reagent Development R&D group in developing amplification reagent and in enzyme improvement.

2003-2004 *Principle Scientist, Department of R&D, MJ Bioworks, Inc.*
Lead two R&D groups, Reagents Development and Instrument Analyses, of 10 people (scientists and research associates). Oversaw the planning and execution of a number of reagents development projects. Ensured support to the instrument development effort. Coordinated collaborations with external R&D groups.

2001-2003 *Senior Scientist, Department of R&D, MJ Bioworks, Inc.*
Lead the Reagents Development R&D team of 5 people (scientist and research associates). Oversaw the development and successful launch of several commercial products.

1998-2001 *Associate Scientist/Research Scientist, Department of R&D, MJ Bioworks, Inc.*
Carried out research project developing new technologies that improve the in vitro performance of DNA polymerases. Independently conceived and validated the Sso7d technology as a novel strategy to improve the processivity of DNA polymerase. The development of this idea ensured a strong IP position of MJ Bioworks, changed the R&D direction of the company, and eventually enabled the launch of several commercial products in the subsequent years.

1993-1998 *Postdoctoral fellow, Christine Guthrie lab, Department of Biochmistry, UCSF*
Carried out research project investigating the structure/function relationship of an RNA dependent ATPase and putative RNA helicase, Prp16, which is involved in pre-mRNA splicing in yeast. Have applied combined approach of Genetics, molecular biology and biochemistry in achieving the research objective.

1986-1992 *Graduate student, Peter von Hippel lab, Institute of Molecular Biology, Univ. of Oregon.*
Completed a doctoral thesis project that studies the mechanism of rho-dependent transcription termination using combined approaches of biochemical and biophysical analyses. Systematically studied the interactions between purified protein and a large number of oligomer RNAs with designed sequences to elucidate the effects of nucleotide sequence on protein-RNA interactions.

EDUCATION:

1986-1992 Ph.D. in Biochemistry, University of Oregon.
1982-1986 B.S. in chemistry, Beijing University, P.R.C.

AWARDS:

1996-1998 The American Cancer Society (ACS) Postdoctoral Fellowship.
1993-1996 The Damon Runyon-Walter Wintchel Cancer Research Postdoctoral Fellowship.
1991-1992 A Research Fellow of the Institute of Molecular Biology, University of Oregon.

PUBLICATIONS:

1. Yan Wang, Dennis Prosen, Li Mei, John Sullivan, Michael Finney, and Peter Vander Horn (2004) A novel strategy to engineer DNA polymerase for enhanced processivity and improved performance *in vitro*. *Nucleic Acids Research*, **32**, 1197-1207. (http://nar.oupjournals.org/cgi/content/full/32/3/1197)
2. Yan Wang and Christine Guthrie (1998) PRP16, a DEAH-box RNA helicase, is recruited to the spliceosome primarily via its nonconserved N-terminal domain. *RNA* **4**, 1216-1229.
3. Yan Wang, John Wagner, and Christine Guthrie, (1998) Prp16, A DEAH-box splicing factor, unwinds RNA duplexes in vitro. *Curr Biol.* **8,** 441-451
4. Johannes Geiselmann, Yan Wang, Steven E. Seifried and Peter H. von Hippel. (1993) A physical model of the translocation and helicase activities of *Escherichia coli* transcription termination protein Rho. *Proc. Natl. Acad. Sci. USA* **90**, 7754-7758.
5. Yan Wang and Peter H. von Hippel. (1992) E. coli transcription termination factor Rho. II. Binding of oligonucleotide cofactors. Journal of Biological Chemistry 268, 13947-13955.
6. Yan Wang and Peter H. von Hippel. (1992) E. coli transcription termination factor Rho. I. ATPase activation by oligonucleotide cofactors. Journal of Biological Chemistry 268, 13940-13946.
7. Steven E. Seifried, Yan Wang and Peter H. von Hippel. (1988) Fluorescent Modification of the Cysteine 202 residue of Escherichia coli termination factor Rho. Journal of Biological Chemistry 263, 13511-13514.

PATENTS:

Issued Patent:

Wang Y. Nucleic acid modifying enzymes.
US6627424 (filed on 8/16/2000; issued on 9/30/2003)

Published patents:

1. Wang Y. Nucleic acid modifying enzymes.
   US20030148330 (filed on 9/27/2002, published on 8/7/2003)
2. Wang Y. Nucleic acid modifying enzymes.
   US20030162173 (filed on 5/30/2001; published on 8/28/2003)
3. Wang Y. Sso7-polymerase conjugate proteins
   US20040081963 (filed on 10/23/2002; published on 4/29/2004)
4. Wang et al. Methods of using improved polymerases
   US20040002076 (filed on 11/27/2002; published on 1/1/2004)
5. Wang et al. Nucleic acid modifying enzymes
   US20040191825 (filed on 4/9/2004; published on 9/30/2004)
6. Wang et al. Parallel polymorphisom scoring by amplification and error correction.
   EP1456409 (published on 9/5/2004)
7. Wang et al. Polymorphism and haplotype scoring by differential amplification of polymorphisoms.
   EP1456395 (published 9/15/2004)